The first two authors contributed equally to the study. Part of this work was undertaken in The Cancer Gene Cloning Laboratory. We would like to thank the patients who took part in this study.

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Limited contribution of interchromosomal gene conversion to *NF1* gene mutation

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EDITOR—Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with a population frequency of 1 in 3500.¹ The disease is clinically characterised by multiple neurofibromas, café au lait spots and Lisch nodules of the iris. The *NF1* gene, a tumour suppressor gene, resides on the proximal long arm of chromosome 17 (17q11.2). It spans approximately 350 kb of genomic DNA and, comprising 60 exons, encodes the protein neurofibromin.² This protein, consisting of 2818 amino acids, contains a central domain that has homology with GTPase activating proteins (GAPs).³

A distinct feature of the *NF1* gene is the very high spontaneous mutation rate (1×10^{-4} per gamete per generation), which is about 100-fold higher than the usual mutation rate for a single locus.¹ Up to 50% of all NF1 cases are thought to result from de novo mutations. The *NF1* gene provides a large target for mutations because of its relatively large size, but this may only account for a factor of 10 in terms of increase in mutation rate.⁴ The presence of numerous *NF1* pseudogenes has been proposed as an explanation for the high mutation rate in NF1.⁵ In the human genome, at least 12 different *NF1* related sequences have been identified on chromosomes 2, 12, 14, 15, 18, 21, and 22.⁵⁻¹³ Most of the *NF1* pseudogenes have been mapped in pericentromeric regions. The chromosome 2 *NF1* pseudogene has been localised to region 2q21, which is known to contain the remnant of an ancestral centromere.¹⁴ Owing to the absence of selective pressure, mutations may accumulate in the *NF1* pseudogenes. Consequently, the pseudogenes

could act as reservoirs of mutations, which might be crossed into the functional *NF1* gene by interchromosomal gene conversion.⁵ Gene conversion, the non-reciprocal transfer of genetic information between two related sequences, has been recognised as a mutational mechanism for several human genes.¹⁵⁻¹⁷ In all these cases, the conversions occurred between gene and pseudogene on the same chromosome. For NF1, interchromosomal gene conversion is required as none of the *NF1* pseudogenes is located on chromosome 17. Interchromosomal gene conversion has been reported to occur between the von Willebrand factor gene on chromosome 12 and the von Willebrand pseudogene on chromosome 22.¹⁸

Gene conversion requires close contact between the functional gene and the corresponding pseudogene. The pericentromeric location of the functional *NF1* gene and its pseudogenes may enable this close contact since centromeres tend to associate with each other in a non-random fashion.^{19,20} This is underlined by our finding that the *NF1* pseudogenes on chromosomes 2, 14, and 22 have arisen by repeated transposition events between (peri)centromeric locations on these chromosomes (Luijten *et al*, submitted).¹³ However, the high mutation rate in NF1 cannot be explained exclusively by interchromosomal gene conversion. Only a small part of the functional *NF1* gene is represented in the *NF1* pseudogenes (see below), while *NF1* gene mutations are scattered over the entire gene. In this study, we investigated whether interchromosomal gene conversion contributes to the mutation rate in NF1.

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Table 1 Publicly accessible *NF1* pseudogene sequences

Chromosome	Variant	Exons	Accession No	Reference
2		10b, 12a-19a, 27b	AF232292-AF232302	13
2		12b-19a, 27b	AC0009477	23
2		13-15*	Y07858	12
2		13	U35697	8
12		16†	U35686	8
12		16, 17	AC024000	Birren <i>et al</i> , unpublished‡
14	A	10b, 12a-19a, 27b	AF232248-AF232258	13
14	B	10b, 12a-19a, 27b	AF232259-AF232269	13
14	C	10b, 12a-19a, 27b	AF232270-AF232280	13
14	D	10b, 12a-19a, 27b	AF232281-AF232291	13
14	E	10b, 12a-19a, 27b	AL512624	Genoscope§
14	F	12b-19a, 27b	AL512310	Genoscope§
14	A, B	13-15*	Y07854, Y07855	12
14		13, 15, 16†, 18	U35696, U35684, U35687, U35690	8
15		13-15*	Y07856, Y07857	12
15		14	AF011743	11
15		15	U35685	8
15		15	AF011746, AF011744	11
15		18	U35691	8
15		19b	U35693	8
15		20-22	M84131	6
15		21	AF011748	11
15		23-1	U35694	8
15		24	U35695	8
15		24	AF011747, AF011745, AF011749	11
15		25-27b	M84131	6
15		13-23-1, 24-27b	AC021585, AC023191, AC037471	Birren <i>et al</i> , unpublished‡
15		13-23-1, 24-27b	AC020679	Birren <i>et al</i> , unpublished‡
15		24-27b	AC060814	Birren <i>et al</i> , unpublished‡
18		7-9, 11	AP001004	Hattori <i>et al</i> , unpublished¶
18		8	U35688	8
18		9	U35689	8
21		7-9, 11	D26141, AC004527	7; Weiss <i>et al</i> , unpublished**
22		10b, 12a-19a, 27b	AC002471, AC003064, AC005374	13
22		13-15*	Y07859	12
22		18	U35692	8

*Sequences of the last part of exon 13, of the complete exon 14, and of the first part of exon 15 have been determined.

†The sequence of only part of exon 16 has been determined.

‡Birren B, Linton L, Nusbaum C, Lander E, unpublished data.

§Genoscope, Centre National de Sequencage (www.genoscope.cns.fr).

¶Hattori M, Ishii K, Toyoda A, Taylor TD, Hong-Seog P, Fujiyama A, Yada T, Totoki Y, Watanabe H, Sakaki Y, unpublished data.

**Weiss RB, Dunn DM, Aoyagi A, Banks L, Duval B, Hamil C, Holmes C, Mahmoud M, Rose R, Stokes R, Stump MD, Yu P, Zhou L, Gitin Y, Nelson J, von Niederhausen A, unpublished data.

First, we inventoried all available *NF1* pseudogene sequences (table 1). These included not only the published *NF1* pseudogene sequences, but also so far unidentified *NF1* pseudogene sequences present in the first draft sequence of the human genome. The latter were detected by performing a BLAST search

using the complete cDNA sequence of the *NF1* gene (<http://www.nf.org/nf1gene/nf1gene.cDNAtext.html>). In a previous study, we elucidated the complete nucleotide sequence of the *NF1* pseudogene on chromosome 22.¹³ Analysis of this sequence showed that sequences homologous to exons 10b, 12a-19a, and 27b

Chromosome

Figure 1 Overview of the exons maximally represented in the various *NF1* pseudogenes. Exons and introns of the functional *NF1* gene are denoted by black and white boxes, respectively. For the *NF1* pseudogenes, black boxes indicate exons that have been sequenced. The maximum length of the corresponding segments of the functional gene of the *NF1* pseudogenes on chromosomes 2 and 14 is denoted by a dashed line. The actual size of the represented segment of the chromosome 12, 15, 18, 21, and 22 *NF1* pseudogenes is indicated by an unbroken line. The multiple variants of the chromosome 14 and 15 *NF1* pseudogenes have the same genomic organisation.

Table 2 Disease causing *NF1* gene mutations congruous to *NF1* pseudogene sequences

Location	Mutation	Reference	Pseudogene	<i>NF1</i> gene in patient— <i>NF1</i> pseudogene‡
Exon 7	910C>T*†	24	ψ <i>NF1</i> -18	TATTTCT(10nt)TGA—TGTTCCTC(10nt) TGA
Exon 7	910C>T*†	24	ψ <i>NF1</i> -21	TGA—TGG
Exon 10b	1466A>G	25	ψ <i>NF1</i> -2	ACAAGAAGCTGT—ACCAGGAGCTGT
Exon 10b	1466A>G	25	ψ <i>NF1</i> -14A/B	AGAAGCTGT—CGAAGCTGT
			ψ <i>NF1</i> -14D/E	GAC(15nt)TGT—GGC(15nt)TGT
Exon 10b	1466A>G	25	ψ <i>NF1</i> -22	GACCTG(12nt)TGT—GGCTTG(12nt)TGT
Exon 10b	1513A>G	26	ψ <i>NF1</i> -14A/B/D/E, ψ <i>NF1</i> -22	ATA(24nt)GAG—ATG(24nt)GAG
Exon 13	2033-2034insC*	27	ψ <i>NF1</i> -15	GGAACCCCCCG—GCAA(ins14nt)CCTTCCCCG
Exon 13	2033-2034insC*	27	ψ <i>NF1</i> -15	GGAACCCCCCG—GCAA(ins13nt)CCCTCCCCG
Exon 13	2041C>T*†	25	ψ <i>NF1</i> -14A/B/D/E/F, ψ <i>NF1</i> -22	ATTTGCTGA—ACTTGCTGA
Exon 14	2288T>G	Fahsold <i>et al</i> , unpublished	ψ <i>NF1</i> -15	GCGG(6nt)ATTGA—GCGG(6nt)ACTTA
Exon 16	2842C>T	25	ψ <i>NF1</i> -14B/D/E	AATACC(21nt)TAA—AAGACCC(21nt)TAA
			ψ <i>NF1</i> -14F	AATACC(21nt)TAA—AAGACGC(21nt)TAA
			ψ <i>NF1</i> -22	AATACC(21nt)TAA—AGACGC(21nt)TAA
Exon 20	3467A>G†	25	ψ <i>NF1</i> -15	AGCGTA—AGCATA
Exon 21	3526-3528delAGA	Fahsold <i>et al</i> , unpublished	ψ <i>NF1</i> -15	GTTACCA(13nt)ACA—G—GGTACTA(13nt)ACA—G
Exon 22	3721C>T†	25	ψ <i>NF1</i> -15	CTAGCTTGA—CTGGCTTGA
Exon 22	3826C>T*†	27	ψ <i>NF1</i> -15	TTCTGA—CTCTGA
Intron 25	4368-1G>T	25	ψ <i>NF1</i> -15	TTTGAATGTTT—TTCTAA7GTTT
Intron 25	4368-1G>T	25	ψ <i>NF1</i> -15	TTTGAATGTTT—TTTAA7GTTT
Exon 27a	4537C>T*†	8	ψ <i>NF1</i> -15	TGA(9nt)AAGATG—TGA(9nt)AA-ATG

*Mutations recurrently found in the *NF1* gene of *NF1* patients.

†Mutations with a pseudogene equivalent as reported in reference 25.

‡Disease causing mutations identified in the *NF1* gene of *NF1* patients and their pseudogene equivalents are indicated in italics; additional mutations in the pseudogenes compared with the functional *NF1* gene are indicated in bold.

are present in the chromosome 22 *NF1* pseudogene. The same exons are represented in the *NF1* pseudogenes on chromosomes 2 and 14. Chromosome 14 contains several *NF1* pseudogenes. Sequence analyses of all exons present in four chromosome 14 *NF1* pseudogene variants and in the one on chromosome 2 have been performed.¹³ The BLAST search yielded two clones each containing an additional pseudogene variant on chromosome 14. In one of them, sequences corresponding to exons 10b, 12a-19a, and 27b are present, while in the other only exons 12b-19a and 27b are represented. The chromosome 2 *NF1* pseudogene was also found among the results from the BLAST search. In the *NF1* pseudogene present in this particular clone, exons 12b-19a, and 27b are represented.

The complete sequence of the chromosome 21 *NF1* pseudogene has been determined (Weiss *et al*, unpublished data).⁷ In this pseudogene, exons 7-9 and 11 are represented. For chromosome 15, three different *NF1* pseudogenes have been reported.^{6 11 12} A fourth locus may be present on chromosome 15, but this could not be substantiated.¹¹ The representation of the chromosome 15 *NF1* pseudogenes starts in intron 12b and ends in intron 27b. These pseudogenes are the only *NF1* pseudogenes known so far with a nearly complete representation of the GAP related domain, which is encoded by exons 20-27a. Three chromosome 15 clones were found as a result of the BLAST search. Only one of them contains sequences corresponding to exons 24-27b, whereas in both the other two clones exons 13-23-1 and 24-27b are represented. The latter two clones contain the same *NF1* pseudogene variant, but differ from the *NF1* pseudogene present in the former. The BLAST search also yielded two unmapped clones, in which exons 13-23-1 and 24-27b are represented. The *NF1* pseudogene present in one of these unmapped clones is, with the exception of two nucleotides, identical to the *NF1* pseudogene variant present in the two clones that have been

mapped to chromosome 15. Considering this and the fact that in both the unmapped clones exons 20-23-1 and 24-27b are represented, we presume these *NF1* related sequences to originate from chromosome 15. A number of exons of the published chromosome 15 *NF1* pseudogene variants have been sequenced. None of the three pseudogene variants that were identified in the BLAST search is identical to the sequences available from the published chromosome 15 *NF1* pseudogene variants. The chromosome 12 *NF1* pseudogene maximally contains exons 12b-23-1,¹² of which only exon 16 has been partially sequenced.⁸ One of the *NF1* pseudogenes that resulted from the BLAST search contains sequences corresponding to exons 16 and 17. The clone containing this pseudogene was derived from chromosome 6. However, alignment of exon 16 of this pseudogene to other *NF1* pseudogenes showed a difference of only one nucleotide with the exon 16 sequence of the chromosome 12 *NF1* pseudogene. Since no indications for a chromosome 6 *NF1* pseudogene exist so far, we assume this *NF1* related sequence to originate from chromosome 12 rather than from chromosome 6. The *NF1* pseudogene on chromosome 18 consists at the most of exons 7-13.¹² Sequences of exons 8 and 9 have been reported.⁸ The BLAST search yielded an *NF1* pseudogene, mapped to chromosome 18p11.2, in which exons 7-9 and 11 are represented. An overview of the exons represented in the various *NF1* pseudogenes is given in fig 1 and all accessible *NF1* pseudogene sequences are listed in table 1. When compared to the functional *NF1* gene, substitutions, deletions, and insertions are present in all *NF1* related sequences.

We continued by collecting all point mutations and/or minor lesions in the *NF1* gene. In total, we found 225 different disease causing mutations in exons 7-9, 10b, 11-23-1, and 24-27b of the functional gene, the four regions known to be represented in the pseudogenes. These mutations included 30 novel disease

causing mutations that were taken from our own data (Fahsold *et al*, unpublished data; Nürnberg *et al*, unpublished data). Only a very limited number (13 of 225, 5.8%) of these disease causing mutations appears to have a pseudogene equivalent (table 2). In every pseudogene involved, we detected within a short distance (ranging from 2 to 25 nucleotides) from the disease causing mutation at least one extra mutation compared with the functional *NF1* gene (table 2). If the 13 mutations had been generated by interchromosomal gene conversion, it is likely that these extra mutations would also have been transposed into the functional gene of the NF1 patients. However, none of them has been identified in the *NF1* gene of these NF1 patients. Of the 13 mutations found, six reside in CpG sites resulting in C to T transitions. About 32% of all single base pair substitutions causing human genetic disease occur in CpG dinucleotides.²¹ As CpGs of the *NF1* coding region are subject to methylation,²² these six C to T transitions are probably the result of spontaneous deamination of 5-methylcytosine rather than of interchromosomal gene conversion. This susceptibility for C to T transitions is substantiated by the fact that the six mutations found in both the functional gene and pseudogenes include four recurrent mutations of the *NF1* gene (indicated in table 2). Taken together, these results imply that the contribution of interchromosomal gene conversion to the high mutation rate in NF1 is, at best, limited.

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A clinical study of patients with multiple isolated neurofibromas

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EDITOR—Neurofibromas are benign nerve sheath tumours of a heterogeneous nature consisting of Schwann cells, fibroblastic elements, and embedded axons. Neurofibromas may occur singly in genetically normal people at any point along the peripheral nervous system. Multiple neurofibromas are nearly pathognomonic for neurofibromatosis 1 (NF1). In patients with NF1, neurofibromas may be congenital and plexiform or, more commonly, may be smaller masses that begin to accumulate around the time of puberty. Cutaneous and subcutaneous neurofibromas may cause considerable cosmetic disfigurement, but rarely result in neurological dysfunction. Conversely, deep seated neurofibromas on peripheral nerves and spinal roots frequently lead to neurological disability. Inevitably, adult patients with NF1 have other stigmata of the disorder with the most common being café au lait spots, skin fold freckling, and Lisch nodules.^{1 2} NF1 is an autosomal dominant disorder with full penetrance and a defined genetic aetiology that shows no evidence of locus heterogeneity.³ Neurofibromatosis 2 patients are rarely found to have one or more neurofibromas.^{4 5}

Recently, we have become aware of a small number of patients with multiple pathologically proven neurofibromas, who have no other stigmata of NF1. Here we report the clinical characteristics and pathological findings of these patients, and propose the terminology “multiple isolated neurofibromas” to describe this rare condition.

Material and methods

The criteria for inclusion in the study were multiple, pathologically proven neurofibromas without other defining features of NF1. Careful family histories were obtained in order to

document other family members potentially affected, extending to all second degree relatives. Each participant underwent a clinical examination by one or more of the authors (PB, MM, MPS), which included a detailed neurological evaluation and inspection of the skin with a Wood's lamp. For patients who gave a positive family history, medical records from the potentially affected family member and tumour specimens were reviewed to confirm the diagnosis. Blood samples were collected and immortalised cell lines created for future studies.

All available histological and immunohistochemical materials from each patient were requested from treating institutions and reviewed centrally by a single neuropathologist (DNL). In all but two patients, two or more distinct tumours from separate procedures were obtained; in patient BNF9 only a single specimen was available and in patient BNF12 two specimens from the same procedure were reviewed. Specimens were also reviewed from the mother of patient BNF2 and the brother of patient BNF10.

Molecular genetic analysis of the *NF2* gene was limited to specimens from patient BNF11 and his affected son and was performed as previously described.⁶ No molecular analysis was performed of the *NF1* locus.

This study was approved by the institutional review board of Massachusetts General Hospital and informed consent was obtained from all subjects donating tissue.

Results

A total of 10 adult probands were identified who met our criteria of multiple neurofibromas without other stigmata of NF1 (table 1). Age of onset ranged from 6 to 53 years (mean 27.8 years). An

Table 1 Clinical characteristics of patients with multiple neurofibromas. Tumour distribution includes both surgically removed tumours and those still in place

Patient	Age (y)	Sex	Age of onset (y)	First symptom	Distribution of tumours	Findings associated with NF1	Tumour pathology (No of tumours)	Family history
BNF1	44	F	35	Painless mass	Brachial plexus and chest (bilateral)	Single CAL, migraine	Neurofibroma and plexiform neurofibroma (2)	Brother, osteosarcoma
BNF2	36	F	18	Abdominal pain	Scalp, spine and pelvis	Learning disability, migraine, single CAL	Neurofibroma (2)	Daughter, four subcutaneous masses Mother, multiple NF without CAL Maternal aunt and brother, multiple NF (not confirmed)
BNF3	39	F	6	Painless mass	Head and neck, spine, extremities, torso	Leg length discrepancy	Neurofibroma and plexiform neurofibroma (5)	None
BNF4	44	F	16	Facial palsy	Skull base, brachial plexus, spine, chest, pelvis, extremities	None	Neurofibroma (2)	None
BNF9	49	F	48	Chest pain	Brachial plexus (bilateral)	Migraine	Neurofibroma (1)	None
BNF10	47	M	33	Spastic gait	Spine	Skin tags	Neurofibroma (2)	Brother, multiple NF
BNF11	65	M	53	Painless mass	Shoulder, chest, arm (all cutaneous)	Three CAL	Neurofibroma (7)	Son, NF2
BNF12	62	F	20	Painless mass	Face, extremities (all cutaneous)	Three CAL	Neurofibroma (2)	Multiple relatives with “tumours”, none confirmed
BNF14	48	F	18	Facial pain	Head and neck (R greater than L)	Single GTC, hypertension	Neurofibroma (4)	None
BNF15	39	F	31	Hip pain	R hip and buttock	None	Neurofibroma with marked collagen deposition (2)	None

F = female, M = male, R = right, L = left, CAL = café au lait macules, GTC = generalised tonic clonic seizure, NF = neurofibromas.